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GENERAL

1. **How is Optimiser™ different from a conventional 96-well plate?**
    - All assay reactions in Optimiser™ microplate occur in the microfluidic channel at the base of each well. Optimiser™ wells simply serve as loading ports for samples and reagents which are subsequently drawn into the microchannels. Optimiser™ microchannels have a significantly higher surface to volume ratio compared to the wells of a traditional plate. This fact, coupled with significant reductions in diffusion distance results in significant improvements in assay kinetics, performance and work flow compared to traditional microtiter plates.
    - We strongly urge users to familiarize themselves with the user manual and to review the FAQ section and application notes to understand the benefits as well as the limitations of Optimiser™ microplates.
2. **What are the main benefits that Optimiser™ offers over conventional ELISA plates?**
    - The Optimiser™ microplate system is an extraordinarily versatile ELISA platform. Optimiser™ is the only microplate that allows users to select the performance benefit that is of most significance to their work:
        - Select the ultra-high sensitive mode of operation to achieve **femtogram level sensitivities** for most assays.
        - Use the ultra-fast mode with only **5 min incubation** for most assay steps (**total assay time less than 90 minutes**) and still achieve comparable sensitivity to conventional ELISA's.
        - All assay protocols on Optimiser™ use **only 5 µl sample volume** allowing you to conserve precious sample.
        - All assay protocols on Optimiser™ use **only 5 µl reagent volume** allowing you to cut total assay cost.
        - *See Application Notes (under the Technical Support Tab) on Siloam's website for more details.*
3. **What are the dimensions of the microchannel?**
    - The microchannels on Optimiser™ are 200 µm x 200 µm.
4. **How much liquid can the pad absorb?**
    - Each pad can hold up to 15 ml total volume.

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PIPETTING & FLOW ON OPTIMISER™

5. **Do I need special pipette to work with the Optimiser™?**
    - No, you can use commonly available pipettes.
    - Multi-channel pipette(s) capable of accurately and precisely delivering 5 µL and 30 µL must be used for transferring liquid into the Optimiser™ microplate.
6. **Is there a particular way of loading liquids in the Optimiser™**
    - Reverse pipetting technique must be used for transferring liquid into the Optimiser™ microplate. Please read through the Pipetting Instruction in the instruction manual.
    - See the Optimiser™ Technology page on Siloam's website for instructional videos on pipetting techniques.
    - We strongly recommend first time users to familiarize themselves to the Optimiser™ microplate system ™ using the Optimiser™ Starter kit (OPS-IL6) or OptiMax™ Evaluation Kit (OPD-IL6).
7. **Almost all pipetting protocols specify users NOT to touch the well surface during pipetting. Why does the Optimiser™ user guide suggest the exact opposite?**
    - In conventional 96-well ELISA plates, if the pipette tip touches the bottom surface of the well, it may physically disrupt some of the bound bio-molecules. In the Optimiser™ all the assay reactions occur within the microchannel. Hence, touching the pipette tip on the loading well of the Optimiser™ has absolutely no effect on the assay performance.
    - For most dispensing steps in Optimiser™ based assays, users are dispensing only 5 µl volumes. If the pipette tip does NOT touch the well surface, the dispensed well volume may "bead" and stick to the end of the tip. The well geometry of the Optimiser™ is engineered to ensure smooth filling of well/microchannel provided the liquid is dispensed steadily and directly on the well surface.
    - See the Optimiser™ Technology page on Siloam's website for instructional videos on pipetting techniques.
8. **How critical is the accuracy of the 5 µl dispense volume?**
    - The Optimiser™ is designed such that the 5 µl volume represents a slight excess compared to the microchannel internal volume. Provided that the dispense volume is greater than 4.5 µl, slight (even up to 10%) dispense volume variations will not affect assay results.
9. **Why must all materials be transferred to the Optimiser™ plate within one minute at each step in the assay procedure?**
    - Optimiser™ incubation steps are from 10 to 20 minutes in length. Longer time to transfer material will cause time difference between each well in incubation, which may affect the assay accuracy.
10. **HELP - I can't get the liquids to flow properly?**
    - Most importantly – please check if your solutions are clear of particulates (e.g. supernatants from cell lysate).
    - **We strongly recommend the use of OptiMax™ buffers with Optimiser™ microplates**. OptiMax™ buffers are specifically developed for and validated for use with Optimiser™ microplates.
    - We have validated Optimiser™ plates with aqueous solutions only. Do not use solvent-containing samples.
    - Do not use reagents containing ANY surfactants with Optimiser™ microplates.
    - Very rarely (<0.2%), you will see that liquid has not drained out even after ~ 10 minutes. This indicates a flow failure and signal from that well should not be used for analysis.

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11. What happens if there are differences in the flow rate (time required for different wells to empty)?

- Most surface binding reactions in Optimiser™ plates saturate in ~ 5 minutes. The flow sequence of the Optimiser™ is designed such that differences in flow rates of individual wells do not affect the overall assay performance. If the liquid does empty from the well in 10 minutes then the assay results will be valid.
- Please make sure that the incubation times are **at least** 10 minutes.

12. If one well drains in (say) 1 minute and another in (say) 8 minutes, how is it possible that they provide comparable results?

Although it may seem that difference of minutes may have an impact on the assay precision, Siloam has demonstrated with multiple assays that well optimized assays on Optimiser™ easily achieve CV < 6-10%. The minimal effect of flow rate on precision is a combination of multiple factors:

a. On the micro-scale reaction kinetics are vastly different compared to the macro-scale kinetics of conventional 96-well ELISA plate. In microfluidic channels, most surface binding reactions are saturated in ~ 5 minutes. Optimiser™ characterization data shows that up to ~ 75% of peak adsorption is completed in only 10 seconds and assay binding reactions saturate in ~ 5 minute. This is a result of two factors:
   i. The diffusion distances in the microchannel are extremely small (the channel has a cross-section of only 200 µm x 200 µm) hence diffusion is no longer a limiting factor
   ii. The surface area of the microchannel is ~ 1.5 times the surface area at the base of a conventional 96-well ELISA plate. The volume contained in the microchannel is ~ 5 µl leading to ~ 50x higher surface area to volume ratio which allows for extremely efficient binding reactions.

b. Even for the well that drains in 8 minute, the initial section of the microchannel (towards the center) is filled up in ~ 2-3 minutes. Optimiser™ characterization data shows that the first few loops of the microchannel contribute ~ 95% of the optical signal hence even if the last 1-2 loops take significantly longer to fill, their contribution to the signal is almost negligible. Consequently, variations in signal from the last loops have little impact on overall (assay) signal variation.

c. For most reaction steps in the assay sequence (except for sample/standard loading step) the biomolecules are present in vast abundance and the binding reactions are completed extremely quickly. To ensure good precision, it IS recommended that the sample/standard incubation should be 20 min.

d. Finally, the incubation interval (when there is no liquid left in the well) "smooths out" the effect of flow rate variances.

13. Why has the recommended operating volume been changed to 5 µL? I remember seeing 10 µL as recommended volume in earlier version of the FAQ.

- Minimizing the volume helps with improving the precision. When using the 10 µl protocol, there is higher variation in the "time to empty" for different wells on each plate. This is related to the flow rate of the microchannel and larger volume show more net effect on flow duration (and variation of the duration).
- The new 5 µl protocol also reduces the incidences of "slow" or "stopped" flow. With proper pipetting technique and by use of the new protocol, our lab tests show that flow failure rate (well does not empty after 10 minutes) is now less than ~ 0.2%.
- We have verified through extensive assay tests that change from 10 µl to 5 µl does not affect the assay sensitivity. This is partly owing to improvements made to the OptiMax™ buffer formulations.

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REAGENTS & SAMPLE

14. Why do I have to screen for coat buffers? I have never done that for my conventional plate.

- The assay sensitivity can vary as much as 10x depending on the coat buffer used for capture antibody coating. This additional assay optimization step is critical for Optimiser™ microplates to achieve best performance.
- **OptiBind™ COAT BUFFER IS MANDATORY FOR OPTIMISER™ ASSAYS - DO NOT USE ANY OTHER COAT BUFFER.**

15. What about any requirements for other assay buffers or reagents?

- **We strongly recommend the use of OptiMax™ buffers with Optimiser™ microplates.** OptiMax™ buffers are specifically developed for and validated for use with Optimiser™ microplates.

16. I already have SAv-HRP in my lab; why do I need OMR-HRP?

- Optimiser™ is an exquisitely sensitive ELISA platform and use of appropriate SAv-HRP is critical to ensure good results. OMR-HRP has been characterized for purity, concentration and HRP activity and validated for use with Optimiser™ microplates.

17. What chemifluorescence substrate should I use?

- Siloam's OptiGlow™ Substrate is currently available for use with HRP based assays.
- For Alkaline Phophatase (AP) based assays: AttoPhos® AP Fluorescent Substrate System, Promega, Cat# S1000 may be used.
- Substrates with wavelength less than 500 nm (emission light) are not compatible with Optimiser™.

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READER

18. Do I need a special reader to detect signals from the Optimiser™?

- No. 96-well fluorescence or multi-mode (with fluorescence) plate readers can be used to read Optimiser™ plates.
- *Note:* Absorbance (colorimetric) readers **cannot** read Optimiser™

19. Is the setup for a fluorescence reader different from my absorbance reader?

- Yes, a fluorescence reader requires the user to define wavelengths, gain settings (sensitivity) and plate type. Please read through the Reader Setup in the user manual.

20. Will Optimiser™ plates leak any fluids (from the channel) into my expensive reader?

- No. We have tested Optimiser™ exhaustively and have never seen liquid leak out from the channel. The only precaution we would suggest is to be careful when positioning the plate in the reader – ensure that you do not drop it in the reader tray.

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OPTIMISER™ BASED ELISA

21. **Seriously – I can use only 5 µl sample volume and STILL get the same response?**
    - YES! Optimiser™ microplates are extremely sensitive and efficient platforms for ELISA. The high surface area (50% higher than base of a conventional well) and the very high surface area to volume ratio (50 **times** higher than conventional ELISA plates) allow for extremely efficient binding reactions.
    - This is true for all microfluidic devices and Optimiser™ delivers the **Power of Microfluidics™** to the 96-well microplate architecture.
    - Did you know that in a conventional microplate, less than 5% of biomolecules in solution end up linked to the well bottom? Optimiser™ characterization data shows that more than 30% of biomolecules in Optimiser™ bind to the walls!

22. **Can you suggest a starting point for immunoassay transfer from my conventional 96-well plate to the Optimiser™?**
    - Please refer to the "Assay Transfer Guide" which presents a detailed method description to migrate an assay from conventional 96-well plates to Optimiser™.

23. **What detection modes can use with the Optimiser™?**
    - The current version of Optimiser™ is well suited for fluorescence/chemifluorescence mode detection.
    - We will have a version suitable for chemiluminescence mode available in the near future.
    - Absorbance mode does NOT work with the Optimiser™.

24. **I use a conventional (absorbance) reader for my ELISA; I know that I will need a fluorescence reader but what other changes will I need for the ELISA itself?**
    - Nothing! Most absorbance (colorimetric) assays use an HRP label for the detection antibody to quantify assay signal. All you need is the OptiGlow™ substrate which uses the same HRP enzyme to generate a chemifluorescence signal.

25. **I am not sure if the 10 minute incubation in room temperature will work – I would prefer to incubate for at least 30 minutes or in 37oC. Is that OK?**
    - Actually – NO. We strongly recommend that incubation times should not exceed 20 minutes. Incubating beyond 20 minutes or at 37 ^{o}C, will cause evaporative losses.

26. **How critical is the 10 minute incubation window?**
    - Most binding reactions on Optimiser™ microplates saturate in ~ 5 minutes. Users can actually use even 5 minute incubation steps (except for sample which should be at least 20 minutes). The Application note section (Technical Support Tab) of Siloam's website has an article that describes this in greater detail.
    - We recommend that you start with at least 10 minute incubation cycles/step – but you can certainly use longer (up to 20 minutes) incubation steps. This may be useful when you are processing multiple Optimiser™ plates in parallel.
    - All incubation steps must be at least 5 minutes (at least 20 minute for sample). Incubation steps should not exceed 20 minutes.

27. **Can I use cell lysate supernatants or other biological fluids such as serum or urine?**
    - The flow does work in some circumstances even with particulates in the solution. However, we have seen that the flow is not very repeatable. For these fluids, we recommend using supernatant after centrifuging at 13,000 g for 10 minutes or pass through 0.2 µm filter.
    - Whole blood cannot be used as sample in Optimiser™.

28. What are the possible sources of error?

- Pipetting error, such as mis-loading or injecting, inadequate loading or injection, liquid residue on the outside of pipette tip, etc.
- Bubbles or particles in solution may impede reagent flow.
- Residue on bottom of plate not completely wiped out before reading
- Longer than recommended incubation step

29. How does wiping the plate at the end of the flow sequence help?

- Wiping removes the slight liquid volume spread on the tape. Especially if this is some of your excess substrate mixed with even a small volume of the detection antibody solution, it will lead to large background signal. Wiping eliminates this effect.

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OPTIMISER™ BASED ELISA - GENERAL

30. Can I use Optimiser™ plates for all the assay types that I run on a normal 96-well plate?

- The current version of Optimiser™ has been validated for ELISA applications, such as direct, indirect, sandwich and competitive immunoassays. For other uses, please discuss your application with Siloam tech support.

31. Does Optimiser™ exhibit "edge effects" like a conventional 96-well plate?

- No. The edge effect is really pronounced at high temp incubations and since the incubation on Optimiser™ plates is very quick even at room temp; we have not seen any edge effects in Optimiser™ plates.

32. I have used clear-bottom 96-well plates for fluorescence detection but they have black sidewalls; or the plate is made of completely black material. Optimiser™ is completely clear – won't it have a very high crosstalk between wells?

- Actually – NO. There is no crosstalk between Optimiser™ wells.
  The reason conventional plates have a black sidewall or completely black material is to minimize the ***autofluorescence*** which leads to increased background noise. All fluorescence readers read one well at a time so there is no signal from the adjacent well (since the fluorescent entity in the adjacent well is not being excited).
  The tapered profile of the loading well also helps in directing the excitation light towards the channel underneath the well rather than to adjacent channels.

33. The well is empty a few minutes after I load the substrate – where is the signal coming from?

- The substrate is actually drained from the well but contained within the microfluidic channel at the base of the well. Since all the assay reaction steps have occurred on the wall of the microchannel; the substrate will react with the label on the microchannel wall; and the signal originates from the substrate volume in the microchannel.

34. How can I improve sensitivity of my assay using Optimiser™?

- In most cases, using the assay optimization protocol described in the "Assay Transfer Guide" you should be able to achieve slightly better sensitivity.
- A guaranteed method to significantly increase sensitivity is the use of repeat load process for sample/standard steps. Please see the Application Note (under the Technical Support Tab) on Siloam's website (authored by BioTek) that describes the use of a Precision automation station to increase assay sensitivity more than 100x! Please discuss your application with Siloam tech support and we can offer more accurate guidance.

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